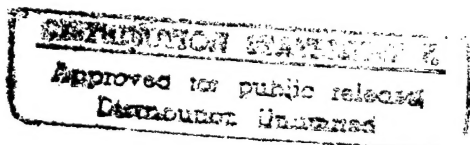


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**CHEMICAL SENSOR FOR MICROSCOPIC MAPPING OF  
SYNAPTIC GLUTAMATE**

ONR # N00014-91-J-1768

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## Grant Objectives

The overall goal of this research project is to develop an analytical procedure capable of measuring *in situ* extracellular glutamate levels in real-time during neurophysiological experiments. Such a system was successfully developed under this grant. This system is capable of measuring extracellular glutamate released from neurons following potassium evoked depolarization.

## Measurement Concept

The concept of the measurement is to place a thin section of brain tissue on the stage of a custom-designed microscope. The tissue is bathed in a perfusion solution that maintains the viability of the tissue and that contains the chemical reagents needed to produce light from glutamate. This light is collected by the microscope objective and directed toward a photon-counting photomultiplier tube (PMT) detector. A burst of light is expected when glutamate is released into the extracellular fluid where the reagents are located.

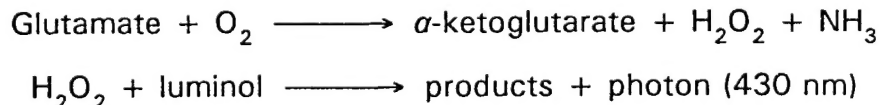
## Results and Accomplishments

### Instrumentation

Figure 1 shows a schematic diagram of the experiment. The perfusion chamber is positioned in the stage of a Zeiss Axiovert microscope and the light produced from extracellular glutamate is collected by an infinity-type objective lens. This light is directed toward a photon-counting detection system and the resulting counts-per-second are recorded by a dedicated computer. The microscope objective provided spatial resolution by collecting photons generated from specific regions within the tissue sample matrix.

### Reaction Scheme

Before the instrumentation could be implemented for glutamate measurements, appropriate chemistry had to be developed to produce light from glutamate. Several potential reaction schemes were evaluated for this purpose and the following biocatalytic/chemiluminescence reaction scheme was deemed most appropriate for synaptic measurements.



First, glutamate is oxidatively deaminated to form  $\alpha$ -ketoglutarate, hydrogen peroxide and ammonia. This first reaction is selectively catalyzed by the enzyme glutamate oxidase. The hydrogen peroxide formed from glutamate subsequently reacts with luminol in the presence of horse radish peroxidase (HRP) to produce several products including light at 430 nm.

This reaction scheme is relatively complex because the rate of photon production depends on many factors such as the concentrations of glutamate, oxygen,

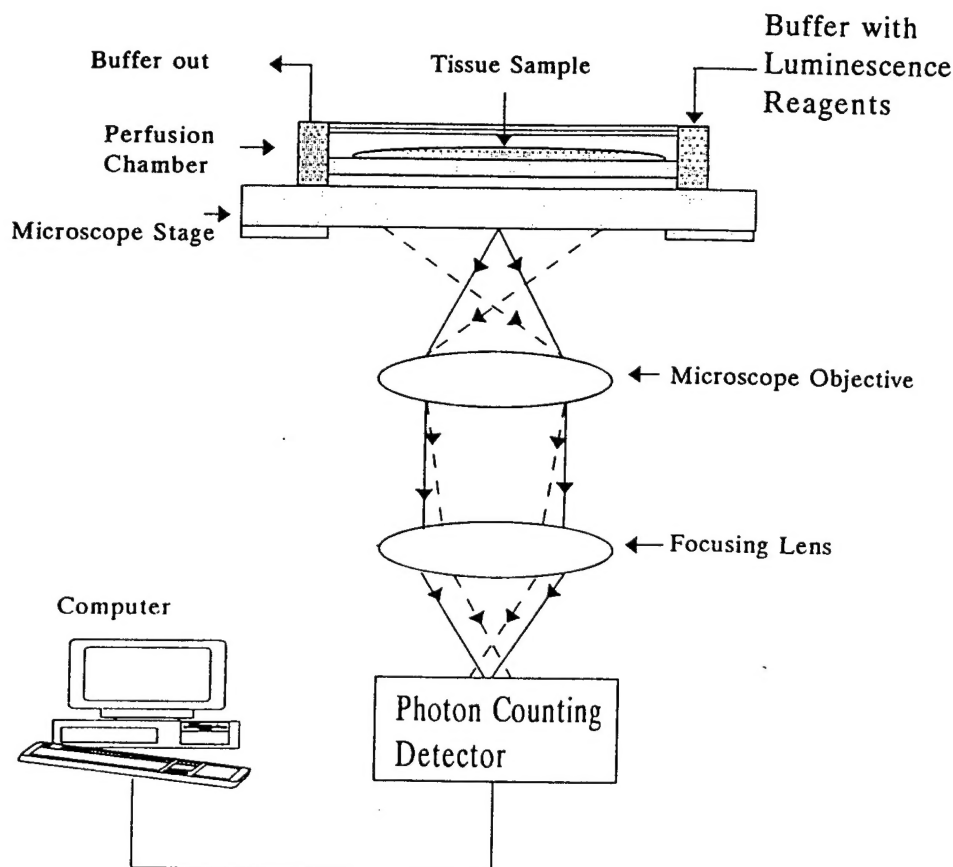


Figure 1. Schematic of glutamate measurement scheme.

luminol, glutamate oxidase, and HRP. We have successfully developed a mathematical model that accurately describes the kinetics of the hydrogen peroxide-luminol reaction component of this scheme. This model is used to establish the ideal reaction conditions for optimal analytical performance, and to identify the sensitivity of the measurement to different chemical species, such as oxygen.

### Intensity-Time Trace

Once the chemistry is combined with the instrumentation, real-time release of synaptic glutamate could be detected. Figure 2 shows a typical intensity-time trace during the potassium evoked depolarization of a hippocampus brain slice preparation. Initially, a low light intensity is measured corresponding to endogenous glutamate levels. A burst of light is then recorded when potassium is elevated in the perfusion chamber and the nerve cells are depolarized. The light intensity reaches a maximum and then decays the initial pre-depolarization level. The initial burst of light corresponds to the sudden release of glutamate and the subsequent decay corresponds to cellular uptake processes and reagent consumption of released glutamate.

The area under such intensity-time curves is related to the number of moles of glutamate released during the experiment. In addition, we can estimate the

concentration of glutamate in the extracellular fluid by comparing the peak height to those obtained from standard solutions of glutamate. Typically, we find extracellular levels of glutamate increases to approximately 12 micromolar following potassium depolarization.

### Effect of Chemical Agents

We have verified that the measured signal tracks extracellular glutamate by evaluating responses obtained under different chemical environments. Figure 3 shows that no signal is recorded when insufficient amounts of potassium or calcium are present. As expected for the measurement of synaptic glutamate, the measured response is calcium dependent. Also, the addition of dihydrokainic acid (DHK) to the perfusion buffer dramatically increases the magnitude of response (data not shown). DHK is a structural analog of glutamate and is known to block high affinity glutamate uptake processes and to enhance glutamate release from neurons. As expected, an increase in light is observed in the presence of DHK due to elevated extracellular levels of glutamate. Finally, many potentially interfering compounds, such as ascorbate and aspartate, have been tested and found to elicit no response.

### TEA-Induced LTP

Exposure to tetraethylammonium ions (TEA) for 7 minutes is reported to induce

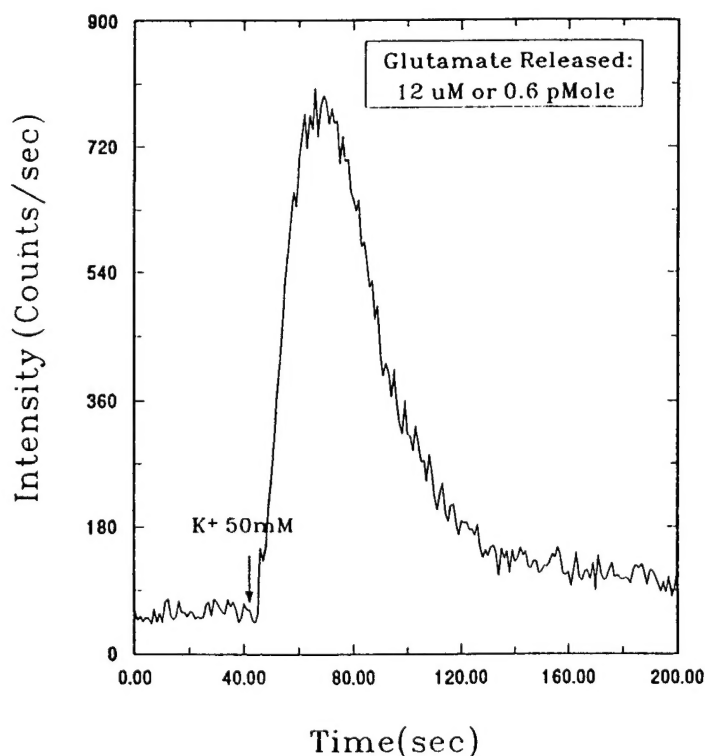


Figure 2. Typical intensity-time trace following potassium depolarization.

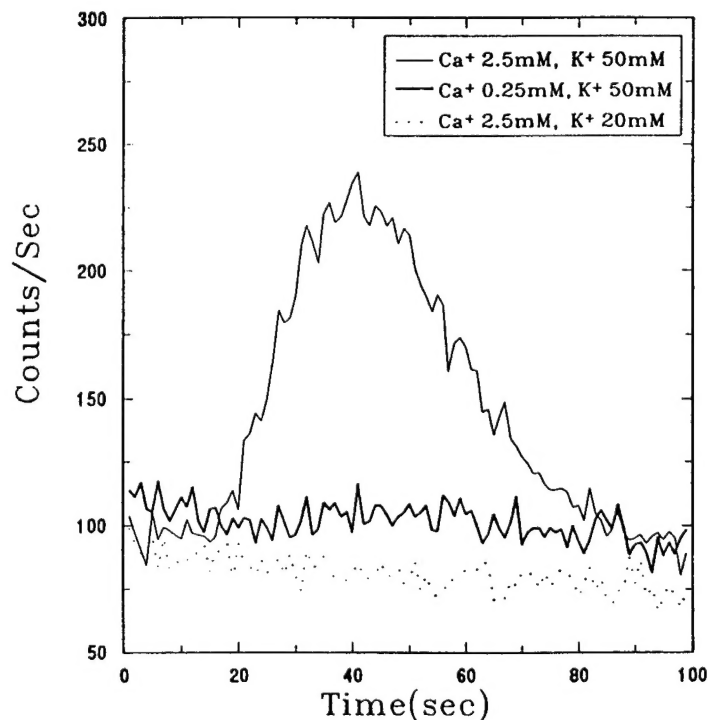
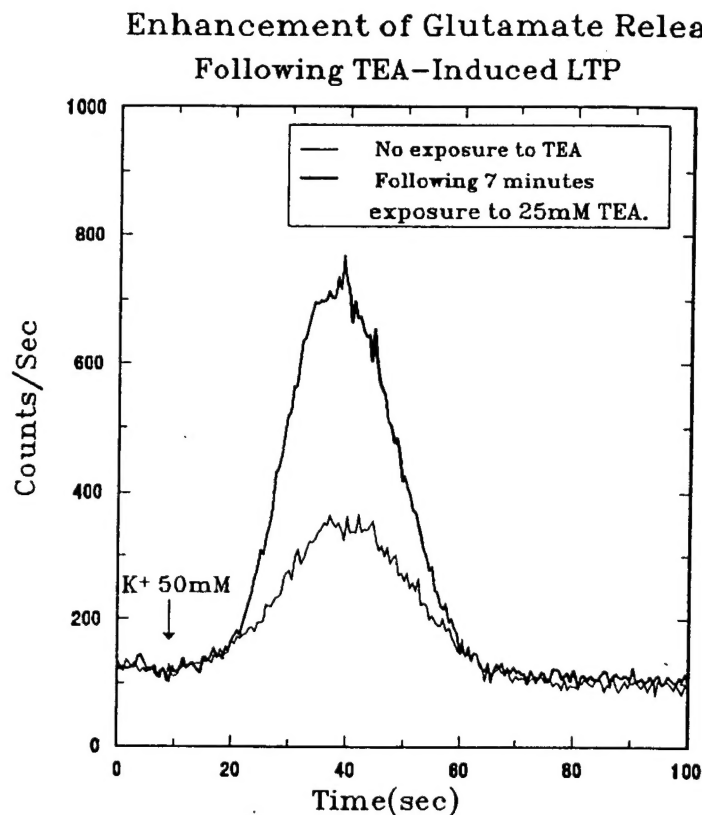


Figure 3. Calcium and potassium dependency on glutamate release.



**Figure 4. Enhancement in extracellular glutamate following chemically induced LTP.**

long-term potentiation (LTP) in hippocampus brain slice preparations (*J. Neuroscience* 1993 **13**, 568-76). We have used our method to monitor, for the first time, differences in extracellular levels of glutamate during TEA induced LTP. Typical results are presented in Figure 4. After a brief 7 minute exposure to TEA, the extracellular glutamate level increases dramatically relative to that measured before exposure to TEA. An increase in extracellular glutamate indicates that, under chemically induced LTP conditions, glutamate release processes are enhanced relative to uptake processes. This finding supports the hypothesis that LTP involves an enhancement in the amount of glutamate released from pre-synaptic cells.

#### Progress and Features

An analytical reaction scheme capable of measuring extracellular glutamate in real-time during synaptic events has been developed. In addition, the instrumentation required to monitor this reaction with brain slice preparations has been designed. Experiments have been performed to establish the feasibility and analytical utility of this novel measurement strategy. This method has enabled, for the first time, a real-time direct look at extracellular glutamate during LTP.

#### **Future Directions**

Several critical and exciting aspects of this project should be pursued as this investigation continues.

1. Although real-time measurements under potassium evoked depolarization conditions can provide valuable chemical information pertaining to synaptic processes, real-time measurements obtained under more physiologically relevant conditions induced by electrical stimulation are desired. Currently, there is no method available for following glutamate transients under electrical stimulation conditions. Results from our initial attempts to use this reaction scheme under such conditions are inconclusive. Although transient signals are observed, we have not been able to verify that these signals are caused by glutamate. The origin of these signals must be identified.
2. An attractive feature of this method is the potential to map the distribution of glutamate within a given tissue slice preparation. The feasibility of this feature has been demonstrated by focussing the microscope objective on different regions within a hippocampus tissue slice. As expected, more light was recorded from the CA1 region relative to either the CA3 or DG regions.

The analytical utility of this approach can be enhanced tremendously by simply upgrading the current PMT detector with a CCD imaging system. A CCD camera will provide a two-dimensional image of the entire tissue slice during a single experiment. Post experimental analysis of the data can be performed to establish the transitory nature of extracellular glutamate levels at any point within the tissue slice. Results can be used to establish different amounts of glutamate released from various regions within the tissue. In addition, differences in the kinetics of glutamate release/uptake can be assessed under identical experimental conditions.

3. Basic research is required to better define the ultimate spatial and temporal resolution capabilities of this method. The ultimate power of this method is the ability to measure in real-time the spatial and temporal distribution of glutamate on a microscopic scale. A firm understanding of the ultimate capabilities of this method requires knowledge that pertains to the overall kinetics of the reaction scheme and the microscopic distribution and mobility of reagents. Experiments are planned to obtain such information.
4. Our analytical system can be configured for other biomedically important analytes such as lactate, glucose, or acetylcholine, by simply changing the oxidase enzyme used in the perfusion buffer. This technology can be used to establish the chemical distribution of these compounds in a wide variety of biomedically relevant systems.

A real-time sensor for nitric oxide is a potential offshoot of our work to measure glutamate by a chemiluminescence reaction scheme. Currently, several chemiluminescence reaction schemes are being characterized as a means to measure *in situ* nitric oxide under neurophysiological conditions.

## Research Presentations

- Arnold, MA; Biosensor designs for *in situ* monitoring; Center for Clinical Pharmacology, University of Pittsburgh Medical Center; Pittsburgh, PA; July 9, 1996.
- Arnold, MA; Optical biosensors for neural active compounds; Department of Chemistry, Ohio University, Athens, Ohio; October 27, 1995.
- Arnold, MA; Chemical sensor technology for neurologically active compounds; Department of Biology, University of Iowa, Iowa City, Iowa; September 8, 1995.
- Arnold, MA; Development of chemical sensors for neurotransmitters; Neuroscience Graduate Seminar Program, University of Iowa, Iowa City, Iowa; October 14, 1994.
- Arnold, MA; Designing selectivity for fiber-optic and electrochemical sensors; Invited speaker in a symposium entitled: Chemical Sensors: A Tribute to Wilhelm Simon; Pittcon94, Chicago, Illinois; February 28, 1994.
- Arnold MA; Development of chemical sensors for real-time analytical measurements; Department of Chemistry, Departmental Seminar, University of Pittsburgh, Pittsburgh, Pennsylvania; September 14, 1993.
- Arnold, MA; Chemical recognition for glutamate selective biosensors; Symposium entitled Chemically Sensitive Interfaces, Division of Colloid and Surface Chemistry, National Meeting of the American Chemical Society, Chicago, Illinois; August 25, 1993.
- Lin, L; Arnold, MA; Dordick, JS; Malinow, R.; Measurement of synaptic glutamate with microscopic photon-counting detection system; Poster presented at the Analytical Division Poster Session at the National Meeting of the American Chemical Society, Chicago, Illinois; August 22, 1993.
- Arnold, MA; Optical sensors for amino acid neurotransmitters; Symposium entitled New Developments in Biochemical Analysis, University of North Carolina at Greensboro, Greensboro, North Carolina; May 1, 1993.
- Arnold, MA; Future prospects for biosensors in clinical and biomedical laboratories; 25th Silver Anniversary, 1993 Oak Ridge Conference on Advanced Analytical Concepts for the Clinical Laboratory, Oak Ridge, Tennessee; April 22-24, 1993.
- Lin, L; Arnold, MA; Dordick, JS; Lian, D; Malinow, R; Measurement of synaptic glutamate with a microscopic photon-counting system; Poster presented at the 8th annual "Survey of Chemical Research in Iowa" Poster Session, Iowa Section of the American Chemical Society, Grinnell College, Grinnell, Iowa; April 17, 1993.
- Li, L; Arnold, MA; Dordick, JS; Measurement of synaptic glutamate by microscopic mapping; paper presented at Pittcon'93, Atlanta, Georgia; March 8, 1993.

Arnold, MA Biosensing based on molecular luminescence and near infrared spectroscopies; Departmental Seminar, Miami University, Oxford, Ohio, February 25, 1993.

Li, L; Arnold, MA; Dordick, JS; Chemistry for the microscopic mapping of synaptic glutamate; paper presented at the 19th annual meeting of the Federation of Analytical Chemistry and Spectroscopy Societies (FACSS), Philadelphia, Pennsylvania, September 21, 1992.

Li, L; Arnold, MA; Dordick, JS; Characterization of the sensing scheme for synaptic glutamate microscopic mapping; poster presented at the Midwest Biotechnology Symposium, Iowa City, Iowa; June 10-12, 1992.

Li, L; Wang, AJ; Arnold, MA; Dordick, JS; Development of a biocatalytic-chemiluminescence reaction sequence for synaptic glutamate mapping; poster presented at the 203rd National Meeting of the American Chemical Society; San Francisco, California; April 5, 1992.

### Publications

Zhou, X; Arnold, MA; Response characteristics and mathematical model for a nitric oxide fiber-optic chemical sensor; *Analytical Chemistry* 1996 68, 1748-1754.

Li, L.; Mathematical model for fiber-optic ammonia sensors and a microscopic photon-counting detection system for the measurement of synaptic glutamate; Ph.D. dissertation, University of Iowa; May 1994.

Li, L.; Arnold, MA; Dordick, JS; Mathematical model for the luminol chemiluminescence reaction catalyzed by peroxidase; *Biotechnology and Bioengineering* 1993 41, 1112-1120.

### Manuscripts for Submission

Li, L.; Arnold, MA; Dordick, JS; Kinetics of acridinium ester hydrolysis and chemiluminescence reactions.

Li, L.; Arnold, MA; Dordick, JS; Microscope photon-counting detection system for the measurement of synaptic glutamate.

Li, L.; Arnold, MA; Dordick, JS; Measurement of *in vitro* glutamate release from rat hippocampal tissue slices with a microscopic photon-counting system.